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13. Abstract ***(Maximum 200 Words)***

The role of oxidative stress in acute laser-induced retinal injury (and other retinal disease/injury modalities) has been long suspected. This study will determine the effectiveness of the snake eye/Scanning Laser Ophthalmoscope model, an *in vivo*, non-invasive imaging technique, for use as a longitudinal model in the study of neural injury/neurodegenerative disease. This represents a significant refinement of the model, which currently provides the unique ability to observe cellular changes in the retina. Progress has been made in three areas of research necessary to establish the snake eye as a model for human neural injury/disease: (1) A study on determining the effect of a single dose treatment with an antioxidant on temporal development of retinal laser injury, including the development of analysis techniques and lesion size quantification. The results indicate that the use of N-Acetylcysteine shows promise as a potential therapeutic tool for this type of injury. (2) The development of ocular electrophysiological techniques in this animal, and function corroboration of the cellar make up of the Great Plains rat snake retina, and (3) an analysis of the photo pigments and biochemistry of snake vision.

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Table of Contents

INTRODUCTION

The objective of this project is to develop an *in vivo* reptilian model for the evaluation of the mechanisms that cause neurodegeneration in the retina following thermal and/or photochemical laser injuries, and to develop a damage model that is applicable to a number of neurodegenerative disorders, such as Parkinson's Disease. Military personnel are often exposed to occupational / operational environments that are putative sources of neurodegenerative processes, e.g., directed energy such as microwaves and lasers. Previous studies (Elliott et al., 1998; Zwick et al., 2000) have shown that degenerative processes due to laser-induced retinal injuries involve primarily reactive oxygen species (ROS) activity. ROS has been implicated as a possible mechanism in Parkinson's Disease (Alexi et al., 2000; Frantseva et al., 2000; Mattson, 2000), which might be the result of a chronic oxidative stress state in the substantia nigra that targets dopaminergic neurons (Grunblatt et al., 2000). Dopamine and its receptors are widely distributed in the vertebrate retina (Ngygen et al., 1999; Djamgoz et al., 1997; Masson et al., 1993), and modulate photoreceptor activity. Djamgoz et al. (1997) have demonstrated that Parkinson's patients show a decrease in dopamine activity in the retina, which results in these patients showing a disruption of contrast sensitivity at higher spatial frequencies and a disruption of electrophysiological activity in the retina as well (Peppe et al., 1998; Ikeda et al., 1994). It is assumed that this retinal degeneration in Parkinson's disease is another manifestation of the same degenerative mechanisms occurring in the substantia nigra, and it is therefore a possibility to use these observed retinal deficits as a quantitative marker for the central degenerative state. In addition to dopamine's contribution to Parkinson's Disease, animal models also implicate cytokine activity and inflammation responses in this neural degenerative process (Grunblatt et al., 2000). This project's objective will include the development of different techniques to demonstrate these processes in an *in vivo* snake eye model. Specifically, the confocal Scanning Laser Ophthalmoscope (cSLO) will be used for morphological evaluations of thermal and photochemical laser-induced damage using the rat snake (*Elaphe g. guttata and Elaphe g. emoryi*). The optical properties of the snake's eye (Figure 1) permit the

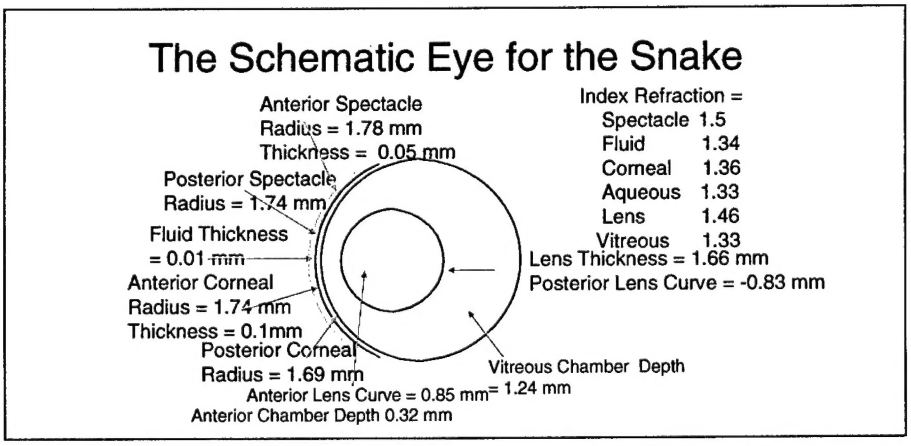


Figure 1: Dimensions and components of the colubrid snake eye: Note the presence of the spectacle; a clear, hard, dry optical first surface. In most vertebrate eyes, the optical first surface is the tear film. Maintenance of the first surface during anesthetized eye exams is critical to image quality, and can be problematic especially in small, wet-eyed animal models.

imaging of the photoreceptor matrix at the single cell level in an intact animal (Figure 2). To capture images of this quality usually requires sacrifice of the animal for histological preparation. Using the snake model, however, only requires placement of the anesthetized subject in front of the cSLO, and therefore this model provides additionally the unique capability of studying retinal changes over time in the same subject (Figures 3 and 4).

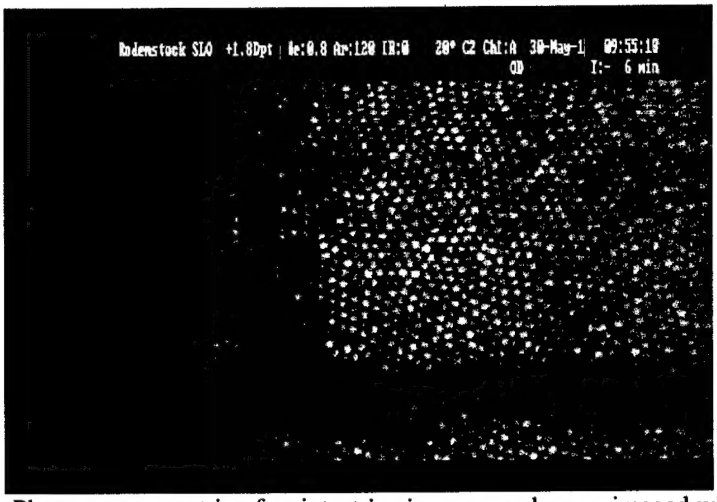


Figure 2: Photoreceptor matrix of an intact *in vivo* corn snake eye, imaged with a cSLO. This all cone retina is similar to the one in the human macula. Cell spacing is ~ 10-12 μ.

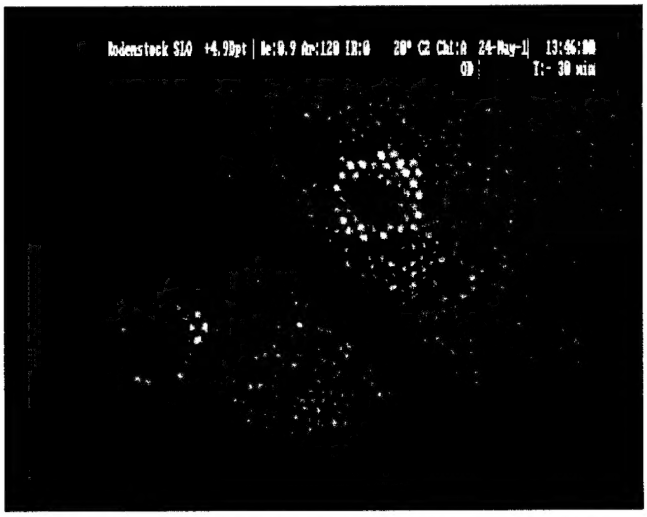


Figure 3: Thermal retinal injury at 1-hr post-exposure. A core of destroyed cells is surrounded by a number of damaged, yet still structurally intact photoreceptors. These are the cells that are the target of a potential treatment

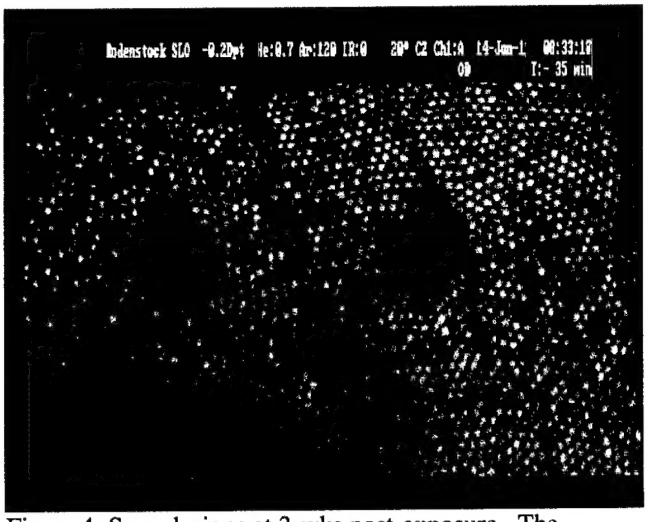


Figure 4: Same lesions at 3-wks post-exposure. The lesions have increased in circumference due to the additional loss of the previously damaged cells that were exposed to oxidative stress.

Using this imaging technique will help to determine if oxidative stress is more prevalent after photochemical or thermal laser exposures. Testing for cytokine expression in the laser injured snake eye will be accomplished via commercially available cellular assay kits, and once identified, vitreous fluid will be sampled continuously from the live animal to construct a temporal description of cytokine modulation after laser exposure. Lastly, visual function will be assessed with electroretinogram (ERG) recordings. These assessment techniques have never been applied to the snake model, and if successful, this *in vivo* model will be used for quantitative studies examining the effects of acute and chronic neurological insults, different modes of insult, and possible treatment regimes.

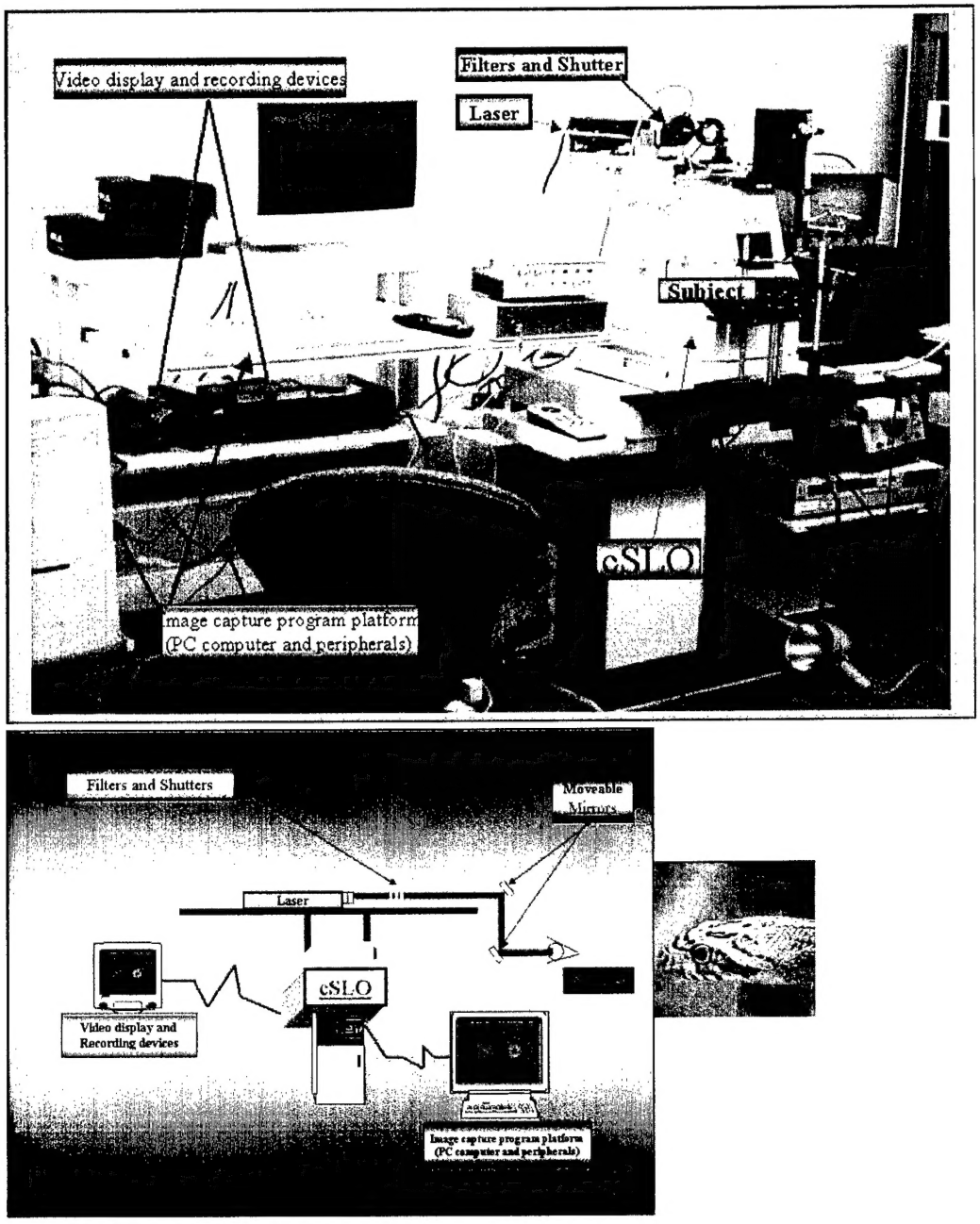


Figure 5: Layout of the bioeffects facility at NHRC DET's Laser Department, Brooks AFB

BODY

Modeling the injury: One goal of this project is to determine how to best model an injury that causes further neurodegenerative processes. After an experimental animal-use protocol was submitted and briefed to the Brooks AFB Laboratory Institutional Animal Care and Use Committee, the morphological changes that occur after producing acute laser-induced retinal injuries were examined. For this purpose, we accomplished two objectives: (1) we replicated the presence of oxidative stress following thermal lesions to the retina using advanced fluorescent dyes and demonstrated that a common antioxidant can halt such reactions; and (2) retinal morphological changes due to thermal laser exposures were evaluated over a three week period. This evaluation was combined with testing a known neuroprotective antioxidant (N-Acetylcysteine) for its ability to reduce the number of retinal photoreceptors lost following exposure to damaging levels of coherent light in an intact, *in vivo* snake model.

In Experiment I, two rat snakes were randomly assigned to one of two advanced fluorescent dye conditions (2',7'-dichlorodihydrofluorescein diacetate (C-399) or 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (C-400). After receiving two retinal suprathreshold laser exposures in either eye, the dyes were administered via intra-cardiac injection 30 minutes post exposure. Retinal images were taken using the cSLO immediately following the lesion and at intervals for 24 hours, when the fluorescence fades. After a 3-week washout period, subjects received additional lesions in the alternate eye. Immediately following the lesion, subjects received a single IP injection of 150 mg/kg N-Acetylcysteine (NAC), followed 30 minutes later by an injection of the same fluorescent dye they were exposed to previously. Retinal imaging was done at the same time intervals as before, with and without a fluorescein filter.

This study replicated the previous finding that ROS are prevalent following laser-induced retinal lesions. Particularly the cells immediately surrounding the initial site of injury were marked by the advanced fluorescent dyes (oxidative stress). Our results, even though qualitative in nature, successfully show the progression of oxidative stress and cell loss over time with both dyes (Figure 6 shows images using D-399, and Figure 7 images using D-400). One interesting observation was that the C-400 dye additionally highlighted the cellular immune response to the laser injury, clearly delineating the white blood cells migrating to the lesion site (Figures 8 and 9). The ability of a single dose of 150 mg/kg NAC to halt the oxidative stress response, is displayed in the images of Figure 10, which show an absence of fluorescence for treated lesions for up to 24-hrs post exposure, and the number of fluorescent cells continue to be lower for treated eyes, as compared to untreated ones until the dye fades.

Failure to find a consistent fluorescent pattern, poor image quality, and still being in a stage of developing techniques, did not allow for quantification of the present results, however, this could possibly be overcome with the use of an alternate dye. Our objective is to be able to quantify these results, because the application of fluorescent dyes could be an additional powerful tool for determining the parameters (e.g., dosage and time of administration) for an antioxidant, such as NAC, to most effectively prevent and/or decrease oxidative stress and further cell loss.

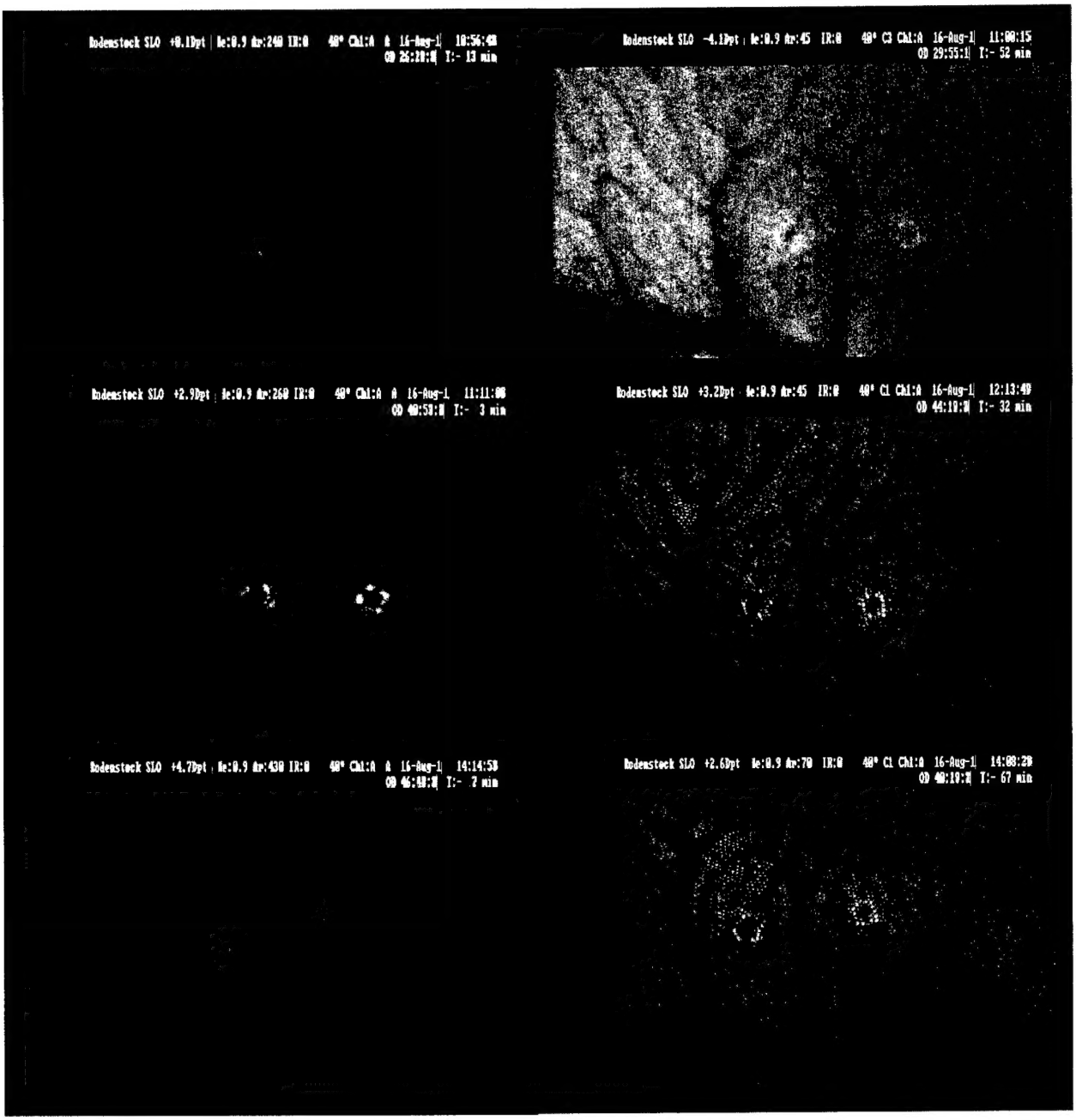


Figure 6: Oxidative stress marked by D-399. Fluorescent images of two lesions are presented on the left. Images of the same lesions taken without a fluorescein filter are presented on the right for comparison. Images were captured (from top to bottom row) at 90-min, 105-min, and at 4.5-hrs post-exposure, respectively.

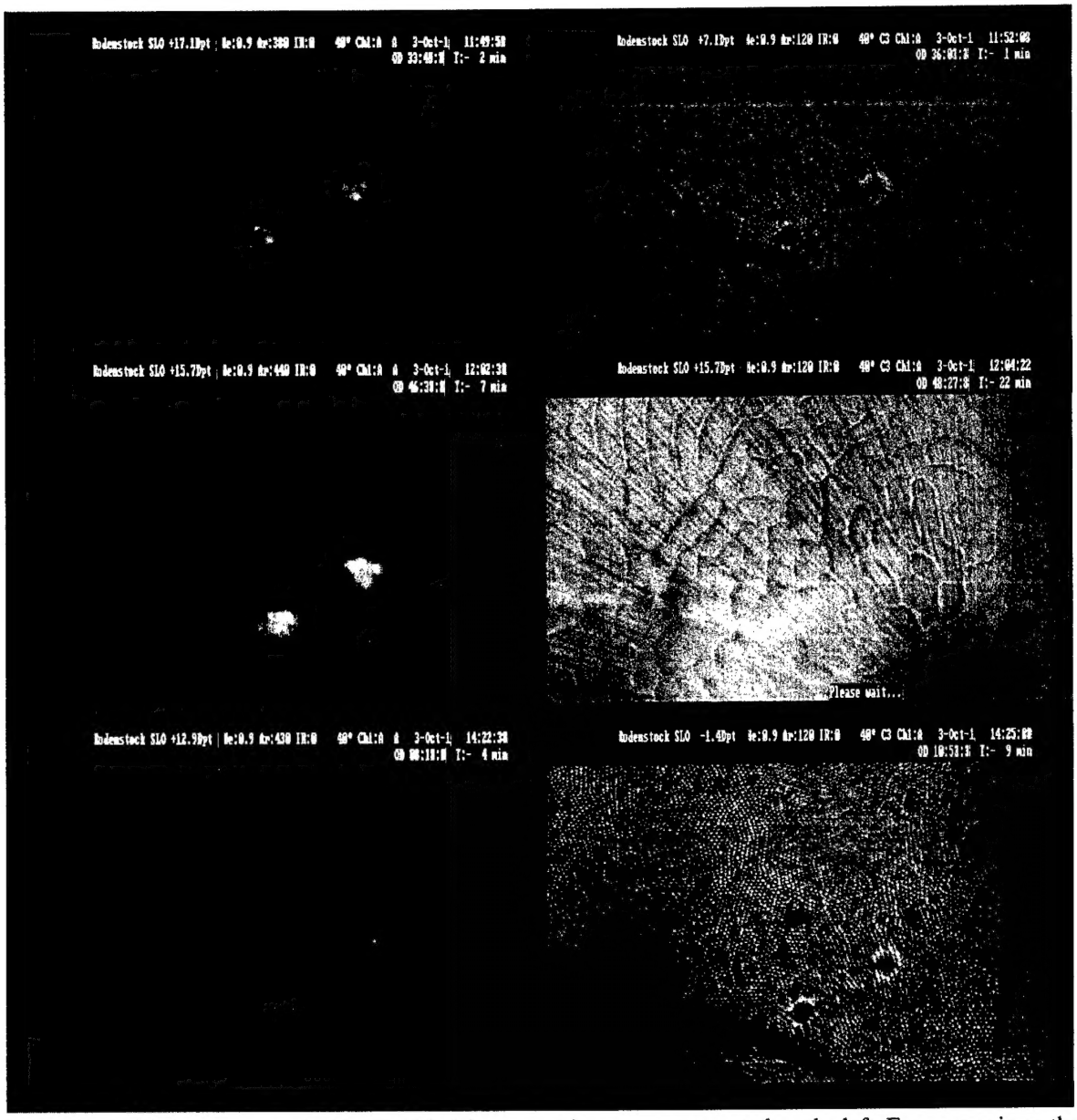


Figure 7: Oxidative stress marked by D-400. Fluorescent images are presented on the left. For comparison, the same lesions are presented in the images on the right and were taken without a fluorescein filter. Images were captured (from top to bottom row) at 75-min, 90-min, and at 4-hrs post-exposure, respectively. Note the presence of two old lesions located just dorsal to the current ones in the 4-hrs post-exposure image taken without a fluorescein filter (bottom right image). These lesions show no sign of any remaining oxidative stress and/or damaged photoreceptors in their immediate vicinity. Note also, that these two lesions are not fluorescent (bottom left image).

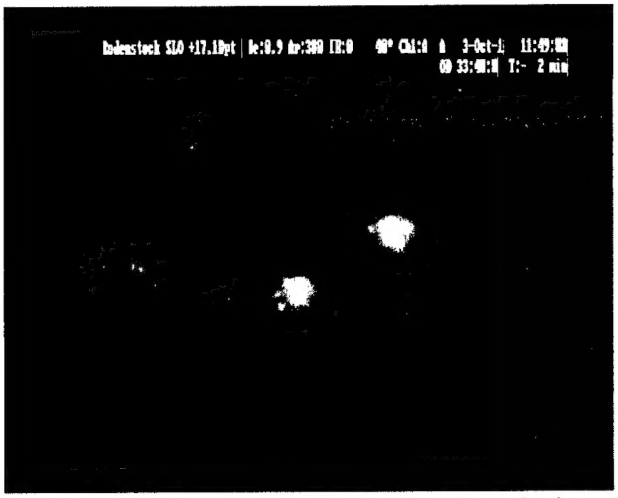


Figure 8: Oxidative stress marked by D-400 at 75-min post-exposure showing a cellular immune response to the retinal damage. Both lesions, as well as leukocytes migrating to the injury site are clearly fluorescent.

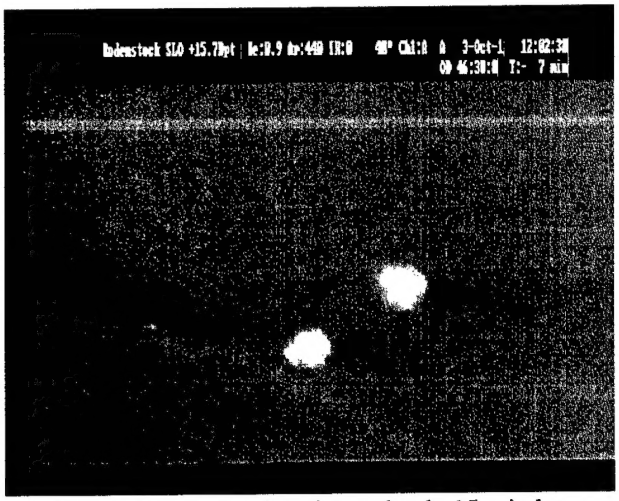


Figure 9: Same two lesions imaged only 15-min later. Lesion site is even more fluorescent and there are fewer leukocytes in transit.

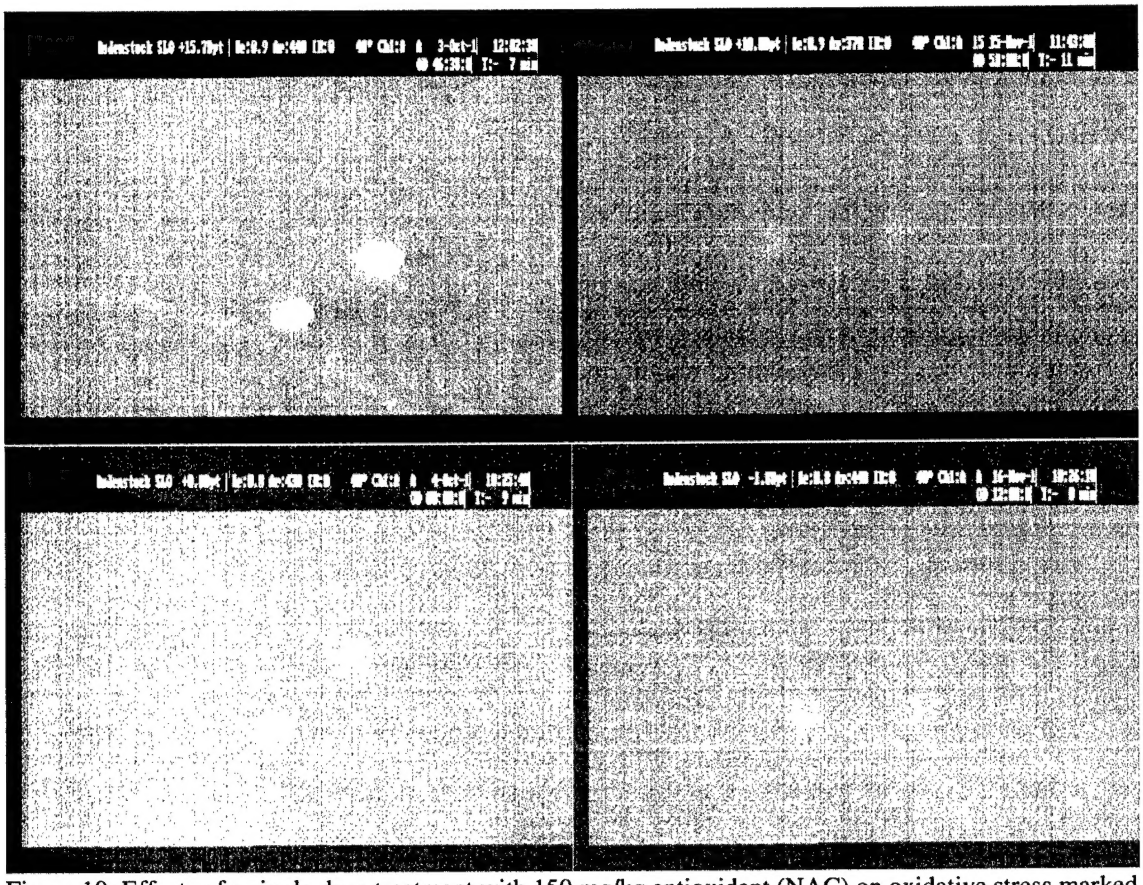
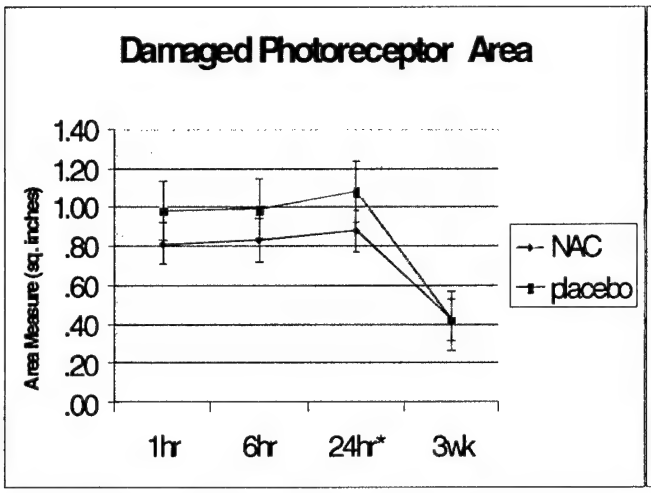
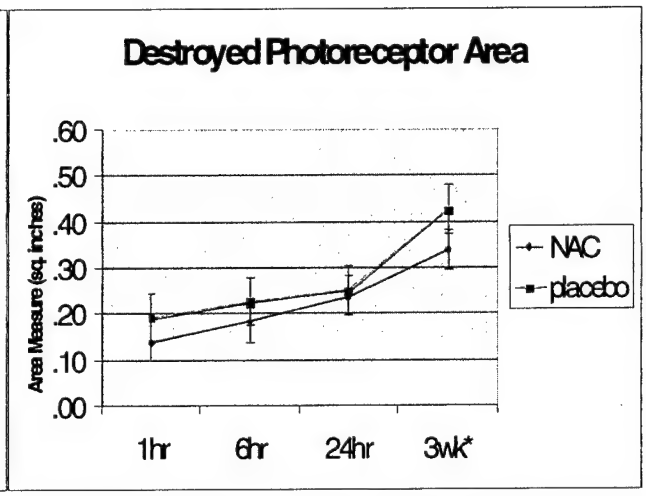


Figure 10: Effects of a single dose treatment with 150 mg/kg antioxidant (NAC) on oxidative stress marked by C-400 at 80-min (top images) and 24-hrs (bottom two images) post-exposure, respectively. C-400 dye images were taken from the same subject; Left: untreated eye; Right: after a 3-wk washout period, lesions in alternate eye were treated immediately post-exposure with 150 mg/kg N-acetylcysteine.

Experiment II was designed to evaluate quantitatively the effects of thermal laser exposures and the effectiveness of antioxidants in preventing further cell loss post-lesion. For this purpose, twelve Corn snakes were randomly assigned to one of two conditions with a varying time between the laser exposure and antioxidant treatment (i.e., immediately following (0 hrs) or 2 hours (2 hrs) post lesion). Additionally, subjects were randomly assigned to one of two treatments (Drug or Placebo). Each anesthetized subject received five suprathreshold thermal laser exposures in a linear array on the inferior nasal retina in a randomly chosen eye. This as well as the previous experiment employed laser exposures that were made with a coaxially aligned Intelite CW Nd:VO_4, DPSS, 532nm laser (shuttered pulses: 20 msec; power: 50 mW; energy per exposure: 1 mJ), each resulting in a spot size of approximately 100 μ. For the 0-hrs Group, the subjects received a single IP injection of 150 mg/kg NAC, or saline (Placebo) 30 minutes post exposure, depending on their initial assignment in the protocol. The snakes assigned to the 2-hrs Group received the same treatment as the 0-hrs Group at 2 hours post lesion. For all subjects, cSLO imaging was done pre-lesion, immediately following the lesion, at 1-hr, 6-hrs, 24-hrs, and at 3-week post-lesion intervals. After the initial 3-week washout period, subjects received additional lesions in the alternate eye with the alternate Drug/Placebo condition but remained in the same time interval (Lesion/Drug) condition. Post-lesion cellular losses were evaluated using the digitized cSLO retinal images of both eyes of each subject, which were enhanced by using the public domain U.S. National Institutes of Health (NIH) Image program. Two experimenters blind to the treatment and interval conditions received all images in a randomized order and were instructed to outline the immediate lesion site as well as the area of damaged cells around the core lesion. These damaged cells are the ones assumed to be lost through apoptotic mechanisms due to the post-lesion activity of reactive oxygen species and cytokine activation, and therefore an increase in the core lesion size is expected. The resultant two area measurements (core area vs. damaged area) for each lesion at each time period were automatically saved by the NIH Image program for each experimenter and data were compared between the two drug conditions for statistical significance. A repeated-measures ANOVA revealed a beneficial treatment effect ($p \leq 0.02$) only when data were collapsed across time of drug administration. Separate analysis of the two different area measurements (core lesion area vs. damaged area) showed that the area of damaged cells treated with NAC was significantly different from the placebo treated lesions at 24-hrs (Graph 1). The area measure of the immediate (core) lesion site was significantly different from the placebo treated lesions at 3 weeks (Graph 2). Both differences were significant at the $p \leq 0.01$ level. Graphs 3 and 4 evaluate these two specific results more closely by lesion position in the array. For both area measures, lesions treated with NAC had smaller initial lesions and fewer cell loss post-lesion. This result would indicate that the use of N-Acetylcysteine or possibly a different antioxidant shows promise as a potential therapeutic tool. Further, there is a general trend for increasing lesion size toward the center of the array (Lesion 3), which might be a further demonstration of the known interaction on lesion development by adjacent lesions.



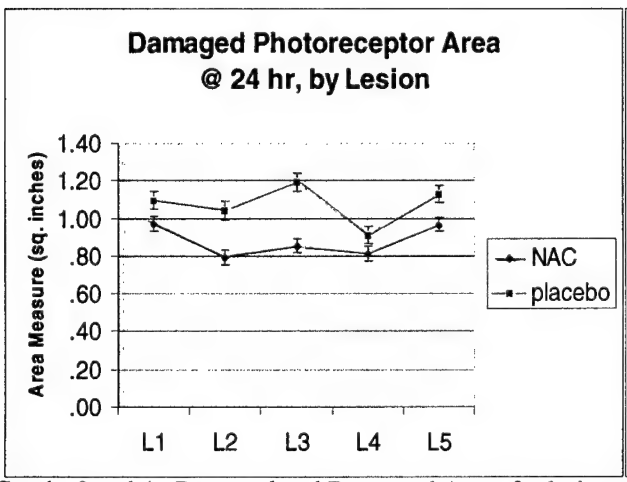
Graph 1: Changes in size of damaged photoreceptor area over time for NAC and placebo treated lesions. Data is collapsed across lesions and treatment time.

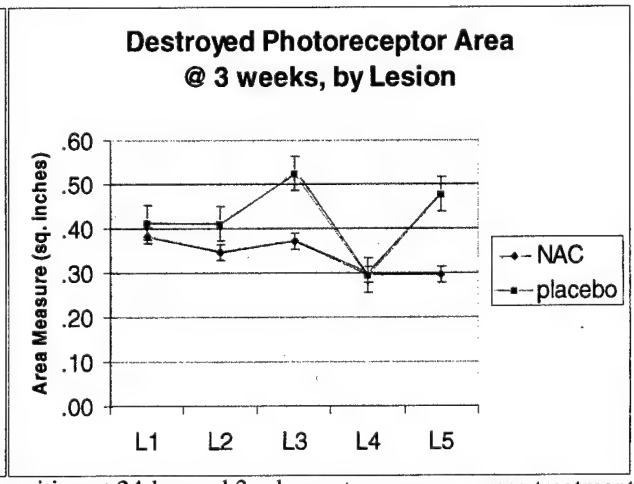


Graph 2: Changes in size of destroyed photoreceptor area over time for NAC and placebo treated lesions. Data is collapsed across lesions and treatment time.

Note 1: An asterisk denotes a significant difference between all treated and all placebo treated eyes at $p \leq 0.01$.

Note 2: An area of 0.40 sq. inches is roughly equivalent to 250 mm^2.





Graphs 3 and 4: Damaged and Destroyed Areas for lesion position at 24-hrs and 3-wks post-exposure across treatment time, respectively. Note the general trend for increasing lesion size toward the center of the array (L3=Lesion 3).

These results demonstrate that the use of the *in vivo* snake model and its retinal images for assessing morphological changes over time and for quantifying photoreceptor damage/loss due to laser exposures is a useful method, however one that will need further refinements. Our study has demonstrated that injections appear to initiate the snake's shedding process and that even though each lesion was produced using the exact same parameters (20 ms shuttered pulse at 50 mW power: 1 mJ energy per pulse), there is a notable variability in the injury outcome. Our results also demonstrate that our method for evaluating the lesions using the digitized images could use further

improvements, because the process of outlining the core lesion as well as the area of damaged photoreceptors is a very time consuming one.

We are currently in the process of developing the parameters needed to produce photochemical lesions in the snake retina, in order to determine which type of insult will yield the best model to study the neurodegenerative processes. Using the same laser and wavelength has proven unsuccessful so far. For the pursuit of this aspect of the study we are currently installing an Argon laser, and we will also try to produce photochemical lesions with a more powerful Q-switched laser that is on order.

Evaluation of chemical changes in retinal tissue in response to acute laser exposures: Several experiments have been completed on the study of retinoids in the snake retina and retinal pigment epithelium, which will help reveal the scotopic vs photopic vision of the snake eye. Briefly, this aspect of the study tries to determine the type and the amount of retinoids in the retina and retinal pigment epithelium of the Corn snake (*Elaphe g. guttata*). Corn snake and garter snake (*Thamnophis*) are both in the family *Colubridae*, which are a crepuscular species with apparently good dim light (scotopic) vision. Their photoreceptors, however, exhibit the morphology of cone (photopic) rather than rod (scotopic) cells. In previous studies, Rodriguez and Tsin (1989) reported that 11-*cis* retinoids (such as 11-*cis* retinyl ester) predominate in the retina and retinal pigment epithelium of cone species. Therefore, analysis of ocular retinoids in the Corn snake may yield novel biochemical insights into scotopic vs. photopic vision. To test this, two animals were light-adapted and anesthetized before eye dissection. Retina and retinal pigment epithelium were isolated under a dissecting microscope. Retinoids were extracted with acetone. Retinyl esters, retinal, and retinol were separated using a five percent water deactivated alumina column before HPLC analyses. Retinoids were identified by retention time and online scan using a photodiode array detector. Quantitation was carried out by comparison with known amounts of authentic standards.

The Corn snake retina had 0.036 nmol of all-*trans* retinyl palmitate/eye (AT-RP), 0.218 nmol of all-*trans* retinal/eye (AT-AL) and no detectable amount of all-*trans* retinol (AT-OL). The snake retinal pigment epithelium had 0.012 nmol of 11-*cis* retinyl palmitate, 0.147 nmol of all-*trans* retinyl palmitate/eye, 0.042 nmol of all-*trans* retinal per eye and no detectable amounts of retinol. Similar results were observed from two animals analyzed separately. Corn snake retina and RPE had a low detectable level of 11-*cis* retinoid and higher AT-RP. Although this finding is atypical in a cone-dominated eye, it is consistent with crepuscular behavior and scotopic vision of the Corn snake. Therefore, it is possible that their photoreceptors have cone morphology but rod-like functions.

Several experiments were also done in order to identify the cytokines that are released in laser-injured snake retina and RPE. Both snake retinas as well as vitreous from the eyes were tested for the presence of cytokines. Beforehand, however, it was necessary to determine whether small samples from the snake's eye contain enough protein for analysis. Our results show that they do (Figure 12).

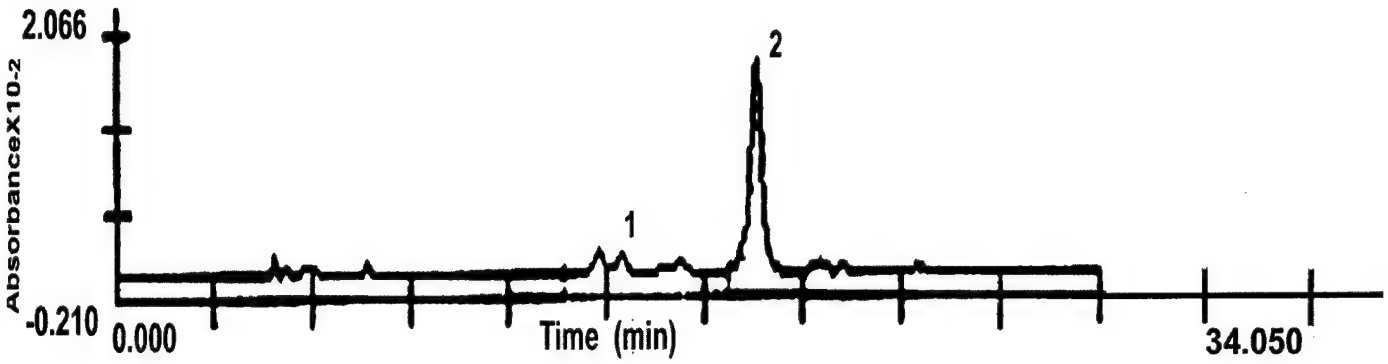
Table 1. Amount of Vitamin A in the RPE and Retina of Cornsnake Measured by HPLC.

	11*cis*-RP	AT-RP	11*cis*-AL	AT-AL	AT-OL
RPE	0.012nmol	0.147nmol	ND	0.042nmol	ND
Retina	ND	0.036nmol	ND	0.218nmol	ND

(Note: Data shown in the table are the per eye average from two observations. 11 cis-RP = 11-*cis* retinyl palmitate; AT-RP = all-*trans* retinyl palmitate, 11 *cis*-AL = 11- *cis* retinal, AT-AL = all-*trans* retinal, AT-OL = all-*trans* retinol and ND = non detectable).

HPLC Tracing of Vitamin A From Cornsnake RPE and Retina

A



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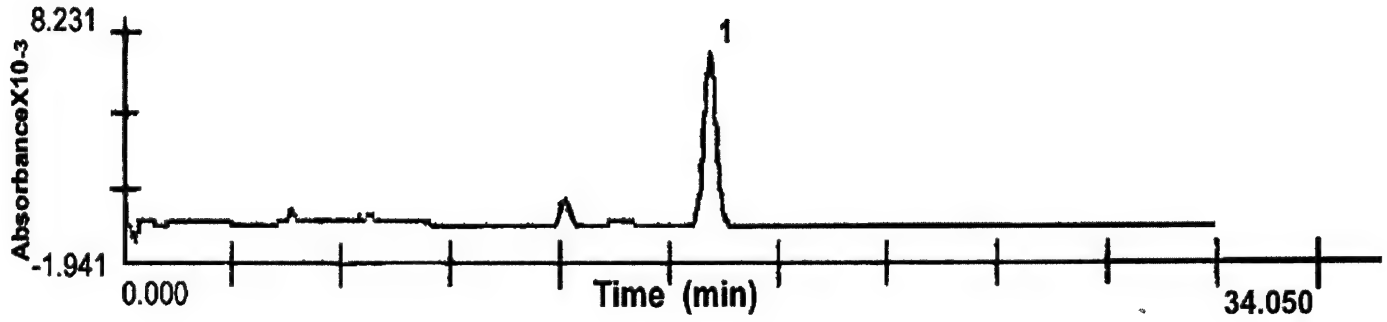

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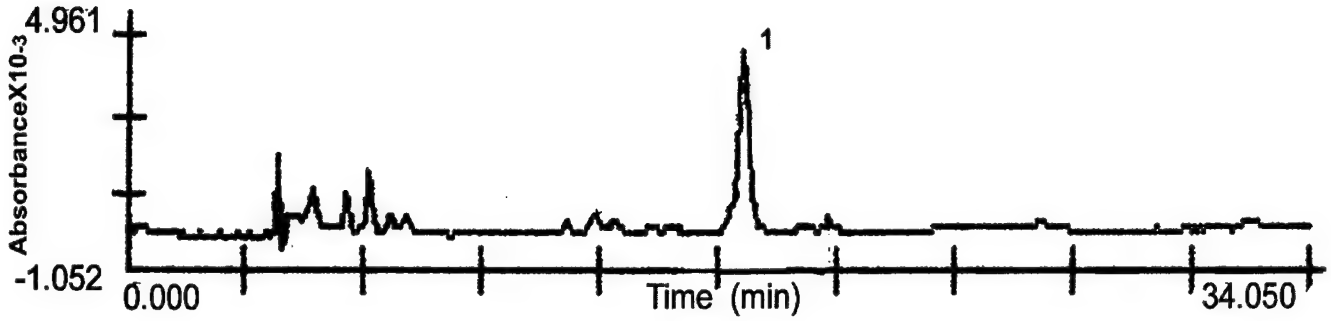

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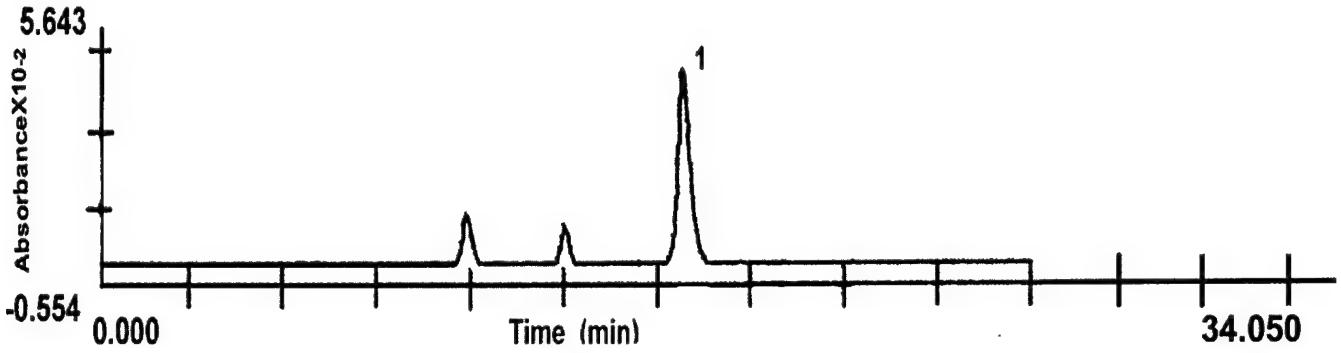

Figure 11 A-D: HPLC Tracing of Vitamin A From Cornsnake RPE (A and B) and Retina (C and D). In the RPE, 11-cis retinyl palmitate (peak 1, RT=13.93min, Fig. 1A) and all-trans retinyl palmitate (peak 2; RT = 17.68min, Fig. A) and all-trans retinal (peak 1, RT = 16.39min., Fig. B) were noted. The retina contains only all-trans retinyl palmitate (peak 1, Fig. C) and all-trans retinal (peak 1, Fig. D).

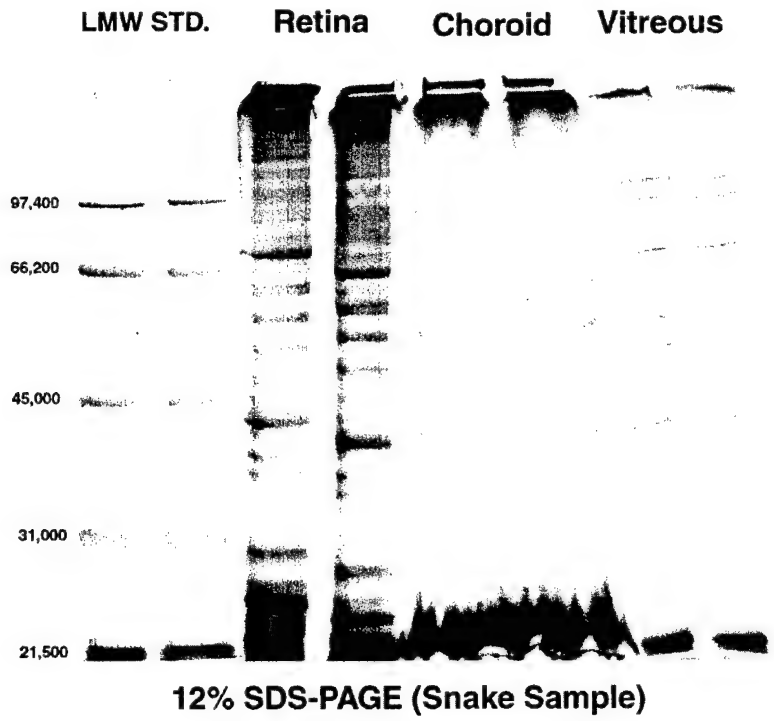

Figure 12: Aliquots of a sample of snake vitreous, retina, and choroids were run on duplicate lanes in the gel, and demonstrate that small samples from the snake's eye contain enough protein for analysis. The two lanes on the far left side are molecular weight standards. Each horizontal band in the gel represents a different protein. There are some bands that correspond in each lane, however, the various samples differ in their protein composition. It appears that the choroid has many more small proteins than the other two sample compartments (note the heavy accumulation of material at the bottom of the choroids lanes).

Preliminary work has established that human and mouse anti-cytokine antibodies that are commercially available do not seem to have sufficient reactivity against the snake cytokines. Therefore, commercial ELISA assays cannot directly be used with snake samples. The approach to be taken will be to utilize bioassays of cytokine function in ocular tissue samples obtained from snakes in terminal experiments following placement of laser lesions in the retina. The first assay to be used will be for platelet activating factor (PAF), which is a common injury- or stress-released factor, and can be done with snake-derived reagents, such as blood cells. Once a tissue fraction containing an active factor is identified, more specific analytical methods, in particular tandem mass spectrometry (MS/MS) analysis of proteins in the fraction, will be used to identify the active cytokine in the fraction, based on sequence homology with known cytokine peptide sequences.

Assessment of functional visual changes using electroretinograms (ERG): The principle accomplishment has been to establish the baseline recording conditions for the ERG from the living snake eye. The major technical problem overcome was to record the ERG from the anesthetized (ketamine/xylazine) snake while leaving the spectacle (a clear, but hard and non-conductive specialized scale covering the eye) intact, so that ocular function (sensitivity to visual stimuli) could be recorded in multiple sessions following laser lesion placement in the retina. The technical solution was to make a small incision in the spectacle near the limbus over the iris, and place a Grass needle electrode into the incision in direct contact with the cornea. A drop of saline was placed on the electrode tip to improve contact. ERG's were evoked by flash stimuli produced by a Grass photic stimulator, set to a mid-level flash. The flash unit was placed about 12 cm from the dark or (background) light adapted eye. Flash rate was varied as indicated. Signals were amplified with a Grass Neuropath Model 12 physiological amplifier with band pass of 1 Hz to 3 KHz, and gain typically of 50,000. Data acquisition was performed with a Data Wave electrophysiology station. ERG waveform included a, b and a small c wave induced by a single stimulus. Flicker ERG results show reduced response amplitude at higher frequency of 22 Hz and almost baseline at 32 Hz (Fig. 13). Using a scotopic background illumination, flicker ERG recording were also obtained. Figure 14 shows that comparable output responses were obtained from light vs. dark-adapted retinas at different stimulus frequency.

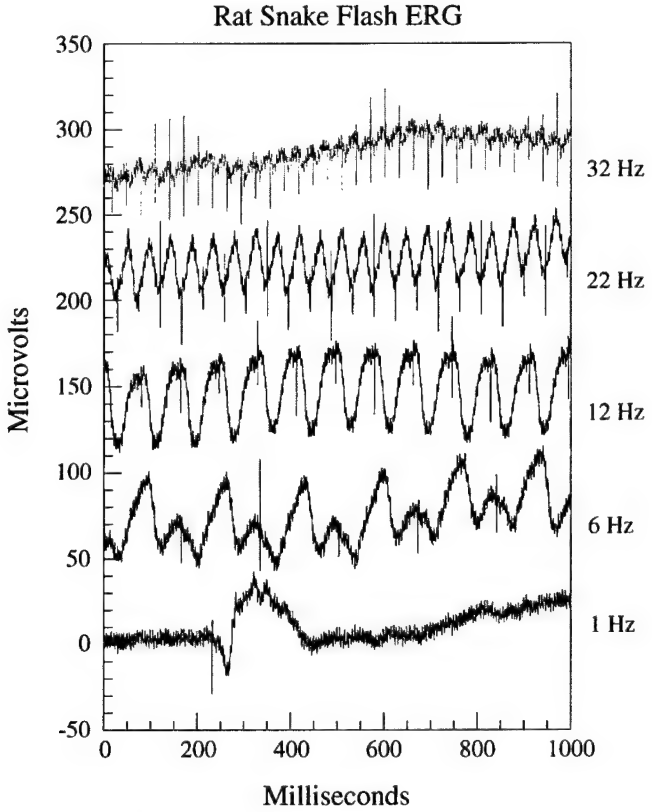

Figure 13: Flash ERG Signal Recorded from a Rat Snake. ERG signal was not averaged, but simply captured as shown. The recorded waveforms were shifted arbitrarily along the amplitude axis for clarity of display.

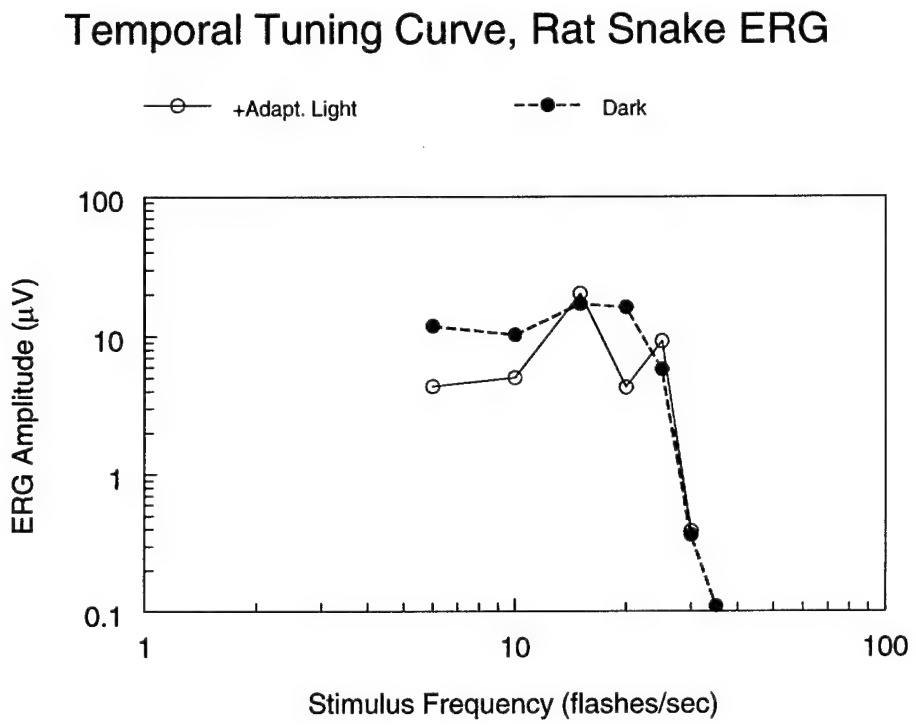

Figure 14: ERG Temporal Turning Curve from a Rat Snake. ERG signals were recorded in dim red light (broken line) and in scotopic level of background light (solid line, to saturate rod response) at different stimulus frequency indicated in the diagram. Amplitudes of ERG's were determined by real-time detection with synchronous lock-in amplifier.

The snake recovers very well from this procedure. The eye does not become infected or desiccated, and it has been possible to perform multiple recording sessions from the same animal. The ERG is acquired and analyzed in real time using synchronous detection, i.e., a lock-in amplifier referenced to the flicker rate of the light stimulus used to elicit the ERG. Flicker ERG responses indicate that recordings were generated from cone photoreceptors. The decline at 30Hz may reflect a difference between warm and cold-blooded species. Comparable ERG amplitudes from light vs. dark-adapted eyes suggest mainly cone functions (rather than rod and cone functions) in the snake retina.

KEY RESEARCH ACCOMPLISHMENTS

- An experimental animal-use protocol was submitted and briefed to the Brooks AFB Laboratory Institutional Animal Care and Use Committee and was approved with minor revisions.
- The finding that reactive oxygen species is prevalent following laser-induced retinal injuries has been replicated, and demonstrated using two different fluorescent dyes.
- This study validated the snake/cSLO model for its effectiveness in assessing morphological changes (in cellular detail) over time, and demonstrated that it is also a useful tool for the purpose of quantifying the extent of retinal damage.
- It was demonstrated that a common antioxidant such as N-Acetylcysteine shows promise as a potential therapeutic tool for laser-induced retinal injuries.
- Experiments are underway to identify the cytokine expression in response to retinal laser exposure (inflammatory response) and several experiments were completed on the study of retinoids in the snake retina and retinal pigment epithelium.
- A method was established on how to do electroretinogram recordings from the live snake to test for visual function changes.

REPORTABLE OUTCOMES

I. Presentations:

- Rentmeister-Bryant, H., Elliott, W.R., Biggerstaff, S. & Zwick, H. (2002). Developing a method for evaluating the protective effects of N-Acetylcysteine on thermal retinal lesions in the colubrid snake eye. Presented at the 23rd Annual Lasers on the Modern Battlefield Conference, Brooks AFB, TX.

- Elliott, W.R., Rentmeister-Bryant, H., Biggerstaff, S., Zwick, H., Hinegardner, M. & Salahuddin, M. (2002). N-Acetylcysteine and acute retinal laser lesion in the colubrid snake eye. Invest. Ophthalmol. Vis. Sci. 43 (ARVO Suppl.):181.

- Leal, R., Tsin, A., Glickman, R., Elliott, R. & Rentmeister-Bryant, H. (2002). Ocular retinoids in cornsnake (*Elaphe g. guttata*). Invest. Ophthalmol. Vis. Sci. 43 (ARVO Suppl.):144.

II. Training supported by this award:

1. Sharon Tang was a graduate student in Engineering. She was supported for three months from 11/1/01-1/15/02. She participated in some of the electrophysiology experiments measuring ERG.

2. Graceila Sable is an undergraduate student in Biology. She is currently performing HPLC analyses of retinoids. She is supported for three months from 5/13/02 to 8/27/02.

III. Funding applied for based on work supported by this award:

Project Title: "Preventative and therapeutic applications of thiol antioxidants in laser eye injuries: Refining the snake eye model for screening of potential treatments."

Funding Category applied for: * 6.2 FY03 Preproposal for ONR 341 BAA 02-009, and
* NHRC IN-House Laboratory Independent Research Preproposal

Funding Result for FY03: Both preproposals were not funded.

CONCLUSIONS

Neural injury and neurodegenerative disease are significant processes in human medicine. Ultimately, the most effective treatment for such conditions will be selected from a myriad of xenobiotic substances. Indeed, the most effective treatments will probably be a combination of substances with synergistic effects. With the need to screen many possible drugs and combinations, an *in vivo* model with the capability to assess cellular level changes could have huge advantages over traditional animal models using serial histology. This model needs fewer animals, requires less euthanasia, and uses a lower order animal than similar experiments with rodents. The snake eye is also one of the few small animal ocular models with an all cone photoreceptor retina, and so may be more applicable to human macular disease/injury than rodent or rabbit models that have rod dominated eyes.

We have established the capability of the Snake eye/cSLO model for longitudinal assessment of cellular changes in the retina due to laser injury. To establish the applicability of this model for neurodegeneration and human disease, we continue to study the snake eye in order to define the differences between snake and human vision.

We continue to test the utility of treatments with substances such as NAC in laser injury, which if proven effective could be used to treat this injury in human accident or battle injury cases with little intervening research. NAC is a well established human pharmacological tool with many clinical uses and no known deleterious side effects.

Baseline conditions for evaluating the ERG have been established. This provides a reference for the work to be done in the upcoming period, in which discrete laser lesions will be placed in the eye. Some fundamental visual physiology has been established for the snakes used in this project (*Elaphe emoryi and Elaphe guttata*). Based on extrapolation from the garter snake (*Thamnophis sirtalis*), which is a very close relative, *Elaphe* has an all-cone retina. The ERG data to date has been consistent with this, showing no difference in the maximum flicker ERG response obtained under dark- and light-adapted conditions.

Finally, this research project has value and significance as basic research, the potential benefits of which cannot be assessed now. Perhaps our understanding of UV cones and their integration in the snake visual system might ultimately lead to the development of better optical sensors, or better camouflage techniques.

REFERENCES

Alexi, T., Borlongan, C., Faull, R., William, C., Clark, R., Gluckman, P., & Hughes, P. (2000). Neuroprotective strategies for basal ganglia degeneration: Parkinson's and Huntington's diseases. Progress in Neurobiology, 60, 409-470.

Djamgoz, M. Hankins, M., Hirano, J., & Archer, S. (1997). Vision Research, 37, 3509-3529.

Elliott, R., Zwick, H., Wood, E., & Stuck, B. (1998). Utilization of acridine orange to characterize leucocyte activity in the snake retinal vascular system. IOVS 39(4):s996.

Frantseva, M., Perez, V., Tsoraklidis, G. Mendonca, A., Adamchik, Y., Mills, L., Carlen, P., & Burnham, M. (2000). Neuroscience, 97, 431-435.

Grunblatt,E., Mandel, S., & Youdim, M. (2000). Neuroprotective strategies in Parkinson's disease using the models of 6-hydroxydopamine and MPTP. Annals of the New York Academy of Science, 899, 262-273.

Ikeda, H., Head, G., & Ellis, C. (1994). Electrophysiological signs of retinal dopamine deficiency in recently diagnosed Parkinson's disease and a follow-up study. Vision Research, 34, 2629-2638.

Masson, G., Mestre, D., & Blin, O. (1993). Dopaminergic modulation of visual sensitivity in man. Fundamentals of Pharmacology, 7, 449-463.

Mattson, M. (2000). Emerging neuroprotective strategies for Alzheimer's disease: Dietary restriction, telomerase activation, and stem cell therapy. Experimental Gerontology, 35, 489-502.

Nguyen-Legros, J., Versaux-Botteri, & Vernier, P. (1999). Molecular Neurobiology, 19, 181-204.

Peppe, A., Stanzione, P., Pierantozzi, M., Semprini, R., Bassi, A., Santilli, A., Formisano, R., Piccolino, M., & Bernardi, G. (1998). Does pattern electroretinogram spatial tuning alteration in Parkinson's disease depend on motor disturbances or retinal dopaminergic loss? Electroencephalography in Clinical Neurophysiology, 106, 374-382.

Rodriguez, K.A. and Tsin, A.T. (1989). Retinyl esters in the vertebrate neuroretina. American Journal of Physiology, 256 (1 pt 2): p. R255-8.

Zwick, H., Elliott, R., & Edsall, P. (2000). A method for *in vivo* detection of photoreceptor oxidative stress induced by laser radiation. In: Protocols in Oxidative Stress, Ed. Don Amstrong. In press.